

Acta Medica Okayama

Volume 39, Issue 2

1985

Article 9

APRIL 1985

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Abstract

Co-cultivation of human thymus and spleen lymphocytes, which were obtained from 26-week and 27-week fetuses, with a lethally-irradiated human cord T-cell line harboring human T-cell leukemia virus type I (HTLV-I) results in the establishment of T-cell lines positive for adult T-cell leukemia-associated antigens and producing HTLV-I. These cell lines had the phenotype of a helper/inducer subset of peripheral T-cells as evidenced by the reactivity with monoclonal antibodies to human T-cells.

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Acta Med. Okayama 39, (2), 155-159 (1985)

— BRIEF NOTE —

TRANSFORMATION OF HUMAN FETAL THYMUS AND SPLEEN LYMPHOCYTES BY HUMAN T-CELL LEUKEMIA VIRUS TYPE I

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Received September 18, 1984

Abstract. Co-cultivation of human thymus and spleen lymphocytes, which were obtained from 26-week and 27-week fetuses, with a lethally-irradiated human cord T-cell line harboring human T-cell leukemia virus type I (HTLV-I) resulted in the establishment of T-cell lines positive for adult T-cell leukemia-associated antigens and producing HTLV-I. These cell lines had the phenotype of a helper/inducer subset of peripheral T-cells as evidenced by the reactivity with monoclonal antibodies to human T-cells.

Key words: human T-cell leukemia virus, human fetal lymphocytes, transformation.

Recently, retroviruses were isolated from human T-cell leukemia and lymphoma independently by two groups. One retrovirus was adult T-cell leukemia virus (ATLV) isolated from patients with adult T-cell leukemia (ATL) (1, 2), and the other was human T-cell leukemia virus (HTLV) isolated from patients with cutaneous T-cell lymphoma (3, 4). These two viruses have recently been shown to be closely related or even the same (5, 6) and are now called human T-cell leukemia virus type I (HTLV-I).

HTLV-I transforms human cord and adult peripheral blood lymphocytes after co-culture with lethally irradiated HTLV-I-producing cell lines (7-9). ATL, a disease with peculiar clinical and geographical features, is mainly confined to adults (10), and ATL cells have the phenotype of terminally differentiated T-cells (11). Therefore, infectivity of HTLV-I to human fetal lymphocytes and phenotypes of transformed cells are interesting subjects to be explored. In the present study, fetal thymus and spleen lymphocytes were successfully transformed by the co-culture procedure.

Thymuses and spleens taken from 26-week-old female and 27-week-old male human fetuses were cut into pieces with scissors, gently squeezed, and passed through 80-mesh screens. Spleen cells were treated with 0.95 % ammonium chloride solution to lyse erythrocytes. Dispersed cells were washed and cultured at 10^6 per ml in 35-mm Petri dishes (3 ml per dish) with RPMI-1640 medium supplemented with 20 % fetal calf serum. After 3 days, 10^6 $10,000$ R-irradiated MT-2 cells were

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added to each culture dish. MT-2 is an HTLV-I-producing T-cell line derived from human cord blood lymphocytes (12) and was used as an HTLV-I source. Thymus, spleen, and irradiated MT-2 cells not undergoing co-culture were used as controls. All cultures were incubated at 37°C in a humidified 5% CO₂ atmosphere and fed twice a week.

In the culture of 26-week-old fetus cells, only co-cultured thymus and spleen cells were transformed, and morphologically altered cells proliferated forming cell clumps about 3 weeks after the co-culture. However, the growth of spleen cells was transient, and only thymus cells were successfully subcultured one month after the co-culture. Thymus cells have since been maintained in continuous culture for over 18 months. In the culture of 27-week-old fetus cells, only co-cultured spleen cells were transformed 6 weeks after the start of co-culture. The spleen cells were first subcultured 13 weeks after being transformed, and have since been maintained in continuous culture for over 6 months. Control cultures gradually degenerated and were never subcultured. The transformed thymus and spleen cell lines were designated as FMT-1 and FMS-1, respectively.

Both cell lines were composed of large lymphoid cells, forming mainly cell clumps intermingled with cells having prickle-like cytoplasmic projections and a few giant cells (Figs. 1a, 1b). Electron microscopy revealed ultrastructural characteristics of immature lymphocytes with peculiar cytoplasmic fibrillar bundles parallel to the plasma membrane. Numerous type C virus particles of variable size, mainly mature, were observed in the extracellular spaces, intermingled with cell debris (Fig. 2).

The results of cell characterization are presented in Table 1. Both cell lines were T-cell lines because they were negative for surface immunoglobulins, positive

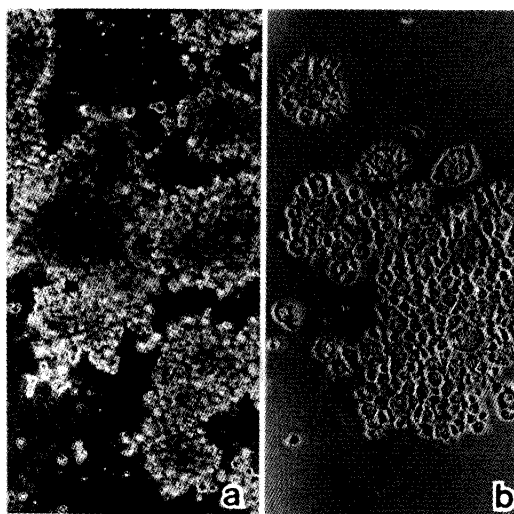


Fig. 1a. Growth pattern of FMT-1 cells. Note the irregularly outlined cell clumps. $\times 57$.

Fig. 1b. Phase contrast micrograph of FMS-1 cells. Cell clumps are composed of lymphoid cells of variable size, some of which have prickle-like projections. $\times 100$.

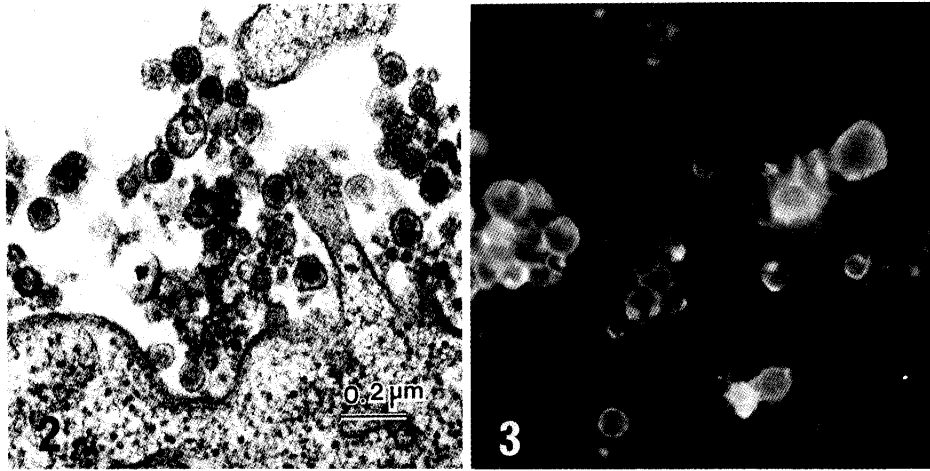


Fig. 2. Electron micrograph of FMT-1 cells. There are many type C virus particles intermingled with cell debris in the extracellular space. They are mainly mature, and the budding form is not seen. $\times 47,500$.

Fig. 3. ATLA of FMT-1 cells. Acetone-fixed cells were first treated with a 1 : 100 dilution of ATL patient serum having an anti-ATLA antibody titer of 1 : 1280 and then reacted with fluorescein isothiocyanate-conjugated goat anti-human IgG. Almost 100 % of the cells are fluorescent, especially in the periphery of the cytoplasm. $\times 615$.

TABLE 1. CHARACTERIZATION OF HTLV-TRANSFORMED HUMAN FETAL CELL LINES

	FMT-1	FMS-1
Gestational age (wks)/sex	26/F	27/M
Organ	Thymus	Spleen
Co-culture	'82-2-8	'82-11-2
Subculture	'82-3-8	'83-3-6
Chromosome	46, XX; 45, XO	46, XY
E rosettes (%)	24	75
Surface immunoglobulin (%)	0	0
Leu 1 (%)	100	100
OKT 3 (%)	17	14
OKT 4 (%)	60	6
OKT 8 (%)	0	0
OKT 11 (%)	100	100
OK Ia 1 (%)	100	100
TdT (%)	0	0
ATLA (%)	100	90
HTLV (%)	+	+
EBNA (%)	0	0

for E-rosetting, and reacted with monoclonal antibodies to pan T-cells, Leu 1 and OKT 11. Furthermore, they reacted with Leu 3, OKT 3 and OKT 4, but not with Leu 2a, OKT 6 and OKT 8. They were also negative for terminal deoxynucleotidyl transferase (TdT) and Epstein Barr virus nuclear antigen (EBNA). ATLA-associated antigens (ATLA) were examined by indirect immunofluorescence using anti-ATLA positive reference ATL patient sera as previously described (1). One hundred percent of the FMT-1 cells and 90 % of the FMS-1 cells were positive for ATLA (Fig. 3). Karyotypic analysis revealed mosaicism of 46, XX and 45, XO in FMT-1 and 46, XY in FMS-1 without structural abnormalities of the chromosomes.

Most human lymphocytes transformed *in vitro* by HTLV-I derived from adult peripheral blood or cord blood have shown the phenotype of a helper/inducer subset of peripheral T-cells as have ATL cells (7-9), although a few cord cell lines have shown a non-T, non-B phenotype (9). FMT-1 and FMS-1, which are of fetal origin, showed the character of terminally differentiated T-cells as evidenced by the reactivity with monoclonal antibodies to human T-cells.

Two possibilities may explain this finding. Lymphocytes having the markers of a helper/inducer subset of mature T-cells might already exist in the fetal thymus and spleen and be transformed by HTLV-I, or immature lymphocytes might be induced to differentiate by HTLV-I infection. Although it can not be determined which explanation is correct, the present study did clearly indicate the susceptibility of T-cell lineage of human fetal tissues to transformation by HTLV-I.

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